

Characteristics of 11 polymorphic microsatellite markers in the red imported fire ant, *Solenopsis invicta* Buren

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Abstract

We have characterized 11 polymorphic microsatellite loci in the invasive ant *Solenopsis invicta*. Primer pairs were evaluated on fire ants collected from monogyne mounds in Lauderdale County, Mississippi. The observed and effective number of alleles ranged from two to six and from 1.31 to 2.64, respectively. The observed and expected heterozygosity values ranged from 0.1613 to 0.7826 and from 0.1491 to 0.6242, respectively. The polymorphism information content of the microsatellites ranged from 0.1482 to 0.6208. Probability tests indicated significant deviations from the Hardy–Weinberg equilibrium at three loci. Pairwise tests did not detect linkage disequilibrium between any pair of loci.

Keywords: fire ant, genetic markers, microsatellite, *Solenopsis invicta*, SSR

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Microsatellites, which are codominant markers, play an important role in population genetics in understanding the role of evolutionary forces such as selection, gene flow, and drift in natural populations. The red imported fire ant (RIFA), *Solenopsis invicta*, is an invasive species of significant economic and ecological importance. The RIFA has emerged as an important case study to investigate the consequences of variation due to place and time, and to estimate the patterns of gene flow (Ross & Keller 1995). Unfortunately, few polymorphic nuclear markers have been available for population genetic studies. Several microsatellite markers for *S. invicta* have previously been reported (Krieger & Keller 1997; Chen *et al.* 2003). Here we report the development of an additional 11 polymorphic microsatellite markers for RIFA.

Genomic DNA of 12 RIFA individuals from a mound collected at Crystal Springs, Copiah County, Mississippi was extracted using a DNA extraction kit for arthropods (Cartagen). A partial genomic library of RIFA was enriched for di-, tri- and tetranucleotide microsatellite sequences using three groups of biotinylated oligonucleotides following a previously described protocol (Perera *et al.* 2007). Biotinylated oligonucleotides of group 1 [(AC)₁₃ and (AGC)₆], group 2 [(AAC)₆, (AAG)₈, (ACT)₁₂, (ATC)₈, (AG)₁₄], and group 3 [(AAAC)₆, (AAAG)₆, (AAT)₆] were hybridized to size-fractionated DNA ligated with an adapter, at 58 °C,

52 °C and 48 °C, respectively. Genomic DNA fragments hybridized to biotinylated oligonucleotides were captured with streptavidin-coated magnetic particles (Promega) and amplified using the SNX-F primer (5'-CTAAGGCCTT-GCTAGCAGAACG-3'). The DNA fragments resulting after two rounds of enrichment were ligated into the pCR 2.1 TOPO cloning vector, and One-Shot Mach1 T1 competent cells (Invitrogen) were transformed with the ligation mix. Recombinant clones were selected on LB agar plates containing 50 µg/mL kanamycin and 40 µg/mL X-Gal. A total of 544 clones were sequenced. SSR Finder software (Sharopova *et al.* 2002) was used to design the primer pairs for amplifying simple sequence repeats containing DNA sequences by following the design criteria outlined in Perera *et al.* (2007). A 'tail sequence' (5'-CAGTTTCCCAGTCAC-GAC-3'), which was identical to the universal fluorescent-labelled primer, was added to forward primers to use a universal fluorescent-labelled primer in genotyping reactions (Taliercio *et al.* 2006). A stabilizer sequence (5'-GTTT-3') was added to each of the reverse primers (Taliercio *et al.* 2006). The sequence analysis and genotyping were performed at the USDA-ARS, Stoneville, Mississippi, using ABI 3730xl instrument (Applied Biosystems). All the amplification reactions were set up to contain final concentrations of approximately 5 ng/µL genomic DNA, 64 nm forward primer, 192 nm reverse primer, 192 nm 6-carboxyfluorescein (6-FAM)-labelled universal primer, 0.1 U/µL of Titanium *Taq* polymerase (BD Biosciences), 200 µM each dATP, dCTP, dGTP, and dTTP, and 1× Titanium *Taq* polymerase buffer

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Table 1 Characteristics of 11 microsatellite loci isolated in *Solenopsis invicta* collected from Lauderdale County, Mississippi

Locus (Accession no.)	Primer sequence (5'-3')	Repeat	Allele size range (bp)	Sample size	H_O	H_E	N_O	N_E	PIC
SiMS1A-43 (EU307210)	F: GCTGCTGTTAAATTGATATTCCG R: GTCGCGTCGAACAAAGTGTAAAT	(AC) ₈	171–174	94	0.4149	0.4967	3	1.9765	0.588
SiMS2A-01* (EU307211)	F: TAGCCTATAAATCAACCGTTGCC R: GCCTTCGTACCTGATTATGCAGC	(AG) ₁₁	178–206	92	0.7826	0.6242	4	2.6372	0.6208
SiMS2A-18* (EU307212)	F: GCTCAAGCACGACAAAGAGAAAAT R: ACTTGGTGATCGACTGTACTGAT	(AG) ₇ AC(AG) ₆	162–187	94	0.4894	0.5586	4	2.2504	0.5556
SiMS2A-50 (EU307213)	F: CTATTAAAGAGCCACTGCACCGAT R: GTTTAATTCTGTTACGCATGAGCC	(TC) ₉	124–132	94	0.2128	0.2406	3	1.3146	0.2393
SiMS2B-65 (EU307214)	F: ATTTTATCGAACGGGAGAAAAAG R: TGCTTCAAAATTACCTTGCAGAAT	(AG) ₄ AA(AG) ₈	157–171	93	0.4409	0.5912	6	2.4274	0.588
SiMS2B-66* (EU307215)	F: ACCGTTGAAATTGAGAAAAACCAA R: AAATGCTTAAAGATCGAGCGACAG	(GA) ₁₃	151–167	94	0.5	0.5354	3	2.1392	0.5326
SiMS3A-39 (EU307216)	F: GCCTTCAAACGCTTCGTATTACA R: TTTGCGATACAAGACCATCGTTA	(ATG) ₄ ATA(ATG) ₇	183–192	94	0.4255	0.3868	3	1.6253	0.3847
SiMS3A-47 (EU307217)	F: AGGGAAAAGGAAAGAGAGCAAGA R: CTCCTCCTGGACTCTCGAACTAA	(AG) ₃ AA(AG) ₉	162–168	93	0.4624	0.5863	3	2.3992	0.5832
SiMS3A-96 (EU307218)	F: TCCGAACAATATCATCCCGTATC R: GGGAAAAGATGTGAGAGAGAA	(TCTT) ₆	118–122	94	0.3723	0.4862	2	1.9367	0.4836
SiMS3B-13 (EU307219)	F: AAATCGACAGCGAGACATTAAAC R: GCGTACGTAACTTTGTGAGTCTT	(TCTT) ₇	138–142	92	0.4891	0.4380	2	1.7720	0.4357
SiMS3B-17 (EU307220)	F: AAGTCTTCTACTTCACATGCAATCT R: TCCGAGATAATTAGGTGTACCAAGC	(AT) ₆	154–156	93	0.1613	0.1491	2	1.1741	0.1482
		Mean	93.36		0.4319	0.4630	3.1818	1.9684	0.4604
		SE	0.0486		0.0455	0.3520	0.1403	0.0452	

Accession no. denotes GenBank Accession number for the sequence from which the primers were designed; 'F' and 'R' denote forward and reverse primer sequences, respectively. Given are the observed number of alleles (N_O), the effective number of alleles (N_E), the observed heterozygosity (H_O), the expected heterozygosity (H_E), the polymorphism information content (PIC) value and the standard error (SE). Loci with asterisk (*) significantly deviated from Hardy–Weinberg equilibrium.

(40 mM Tricine-KOH [pH 8.0], 16 mM KCl, 3.5 mM MgCl₂, and 3.75 ng/μL BSA) in a 5 μL volume. Cycling conditions were: initial denaturation for 2 min at 95 °C, followed by 1 min at 60 °C, and 30 cycles of 15 s at 95 °C, 15 s at 60 °C, and 30 s at 72 °C. A PTC 200 thermal cycler (MJ Research) was used for all PCR amplifications. The PCR products were analysed as 10-fold dilutions on ABI 3730×1 genetic analyser with ROX-500 size standard (Applied Biosystems) and the resulting peaks were scored with GeneMapper (Applied Biosystems) software and confirmed manually.

DNA sequences of 268 unique recombinant clones yielded 92 microsatellite sequences, and all 92 primer pairs were selected for further screening. All monomorphic or stutter-peak-producing primer pairs were eliminated and 11 polymorphic primer pairs that consistently produced single peaks were selected for further evaluation. PopGene version 1.3.1 (Yeh *et al.* 1999) was used for the statistical analyses. Probability tests for Hardy–Weinberg equilibrium (HWE) and genotypic linkage disequilibrium (LD) with sequential Bonferroni adjustment of the P value were performed (Rice 1989). The characteristics of the 11 microsatellite loci in 96 *S. invicta* worker ants, collected from 23 monogyne mounds in Lauderdale County, Mississippi, are

given in Table 1. The observed and effective number of alleles ranged from two to six (average of 3.1818 ± 0.0486) and from 1.31 to 2.64 (average of 1.9684 ± 0.0455), respectively. The observed and expected heterozygosity values ranged from 0.1613 to 0.7826 (average of 0.4319 ± 0.0486) and from 0.1491 to 0.6242 (average of 0.4630 ± 0.0486), respectively. Polymorphism information content (PIC) of the markers was calculated as $\text{PIC} = 1 - \sum P_{ij}^2$, where P_{ij} = frequency of the j^{th} allele for i^{th} locus (Anderson *et al.* 1993). The PIC values ranged from 0.1482 to 0.6208. Significant deviations from HWE were observed for loci SiMS2A-01, SiMS2A-18, and SiMS2B-66. Evaluation of all loci using MICRO-CHECKER software (van Oosterhout *et al.* 2004) did not reveal excessive null alleles at any of the above three loci. Further studies are needed to evaluate if factors such as selection, genetic drift, and non-random mating are responsible for these deviations from HWE. Pairwise comparisons did not reveal linkage disequilibrium between any of the loci.

In the present study, we report 11 polymorphic microsatellite loci that are suitable for extensive population studies of the red imported fire ants. Selection of primer pairs with 60 °C annealing temperatures that produce single-peak

alleles and less than 250 bp amplicons facilitate accurate, high-throughput analysis of populations. Area-wide population genetic studies using these microsatellite markers are underway.

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Microsatellite DNA markers for population-genetic studies of blue mackerel (*Scomber australasicus*) and cross-specific amplification in *S. japonicus*

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Abstract

Blue mackerel (*Scomber australasicus*) is targeted by large-scale purse-seiners in the western North Pacific, and its stock structure is still contentious. Herein, we described 10 polymorphic microsatellite loci for blue mackerel. The number of alleles among 32 individuals surveyed ranged from five to 27 (average of 16.2 alleles per locus). Departures from Hardy–Weinberg expectation were observed at two loci. Cross-specific amplification in the congener, *S. japonicus*, was successful, except for one locus, revealed to be diagnostic for these congeners. These microsatellite loci will be useful tools to address queries in population genetic structure, fishery management unit and taxonomic species status in the genus *Scomber*.

Keywords: blue mackerel, chub mackerel, cross-species amplification, microsatellites, *S. japonicus*, *Scomber australasicus*

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